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The Heme and Sickle Cell Hemoglobin Polymerization

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In search of novel control parameters for the polymerization of sickle cell hemoglobin (HbS), the primary pathogenic event of sickle cell anemia, we explore the role of free heme, which may be excessively released in sickle erythrocytes. We show that concentration of free heme in HbS solutions typically used in studies of polymerization kinetics is ~0.04 - 0.05 mole heme/mole HbS. We show that dialysis of small-molecules out of HbS solutions completely prevents HbS polymerization. We show that that after dialysis, no apo-globin forms and HbS is largely intact. The addition of 100 - 300 μM of free heme to dialyzed HbS solutions leads to nucleation rates and delay times and polymer fiber growth rates faster by two orders of magnitude than prior to dialysis. Towards an understanding of the mechanism of nucleation enhancement by heme, we show that free heme at concentration 66 μM increases by two orders of magnitude the volume of the metastable clusters of dense HbS liquid, the locations where HbS polymer nuclei form. These results suggest that variation of the free heme concentration in the erythrocytes of sickle cell anemia patients may be a major factor for the puzzling complexity of the clinical manifestations of sickle cell anemia. The prevention of free heme accumulation in the erythrocyte cytosol may be a novel avenue to sickle cell disease therapy.

Platform I: Actin & Actin-binding Proteins

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Internal Dynamics of F-Actin Studied By Neutron Scattering

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Actin has a variety of functions related to cell motility. Flexibility of F-actin, a filamentous polymer formed by polymerization of the monomers (G-actin), is important for such multi-functions. Understanding the multi-functions of actin thus requires understanding flexibility of F-actin. Flexibility of F-actin arises from its dynamics, which spans from internal dynamics of the actin-protomer at a picoseconds time scale, through motions of the protomers, to large-scale motions of F-actin at a millisecond time scale. Understanding flexibility of F-actin thus requires characterizing this hierarchy of dynamics. Towards this ultimate purpose, we performed a series of neutron scattering experiments, by which dynamic properties of proteins at pico-to-nanosecond time scale and nanometer spatial scale can be directly measured, of F-actin and G-actin. We performed elastic incoherent neutron scattering (EINS) and quasielastic neutron scattering (QENS) experiments with the spectrometers IN16 and IN5, respectively, at the Institut Laue-Langevin, Grenoble, France. We also performed neutron spin-echo (NSE) experiments with the instrument, iNSE, of the Institute for Solid State Physics, the University of Tokyo, Japan. Analysis of the mean square displacements, estimated from the EINS experiments, showed that G-actin is ''softer'' than F-actin (Fujiwara et al., 2008). Further characterization of the internal motions of the actin-protomers from the QENS spectra showed that these motions have distinct distributions in amplitudes and rates, and the differences in the behavior of F-actin and G-actin arise from the differences in this dynamical heterogeneity. It was also shown that while the motions of G-actin observed by the NSE measurements correspond to translational diffusions, those of F-actin correspond to the relative motions of the actin-protomers within F-actin.

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Molecular Basis for the Instability of Parasitic Actin Filaments

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Apicomplexan parasites such as Toxoplasma gondii use gliding motility to infect host cells, and actin is involved in the regulation of this motility. However Toxoplasma actin, unlike vertebrate actin, does not form long conventional filaments and has been found to have very different polymerization properties. In this work in vitro experiments and molecular modeling are used to elucidate the molecular mechanism behind the unusual instability of parasite actin relative to muscle actin. In vitro observations suggested that the parasite actin are inherently unstable, and stable filaments were only able to be formed with high concentrations of phalloidin. Molecular models of parasite actin filament were constructed and the dynamics were compared with muscle filament actin dynamics to tease the out the differences at the molecular level between these two systems. We found specific amino acid differences gave rise to changes in the interactions between monomers within the filament, and mutation of these residues was able to restore filament stability in experiments. Implications for the structure of the actin filament and the interaction of actin binding drugs will be discussed.

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Hierarchical Crosslinked F Actin Networks: Understanding Structure and Assembly

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The filamentous protein, F-actin provides us with an interesting system in which to investigate the assembly properties of semi-flexible filaments in the presence of cross-linkers and this theme has been explored by several groups. Recently it was observed that F-actin filaments, in the presence of the cross-linker alpha-actinin at high molar ratios are able to generate a novel hierarchical network of filament bundles. We investigate this system using the complementary methods of coarse grained molecular dynamics (MD) simulation, confocal fluorescence microscopy and x-ray scattering.
We have studied the F-actin/ alpha-actinin system in detail with different actin concentrations (Ca) and alpha-actinin-to-actin molar ratios (gamma). The confocal microscopic observations and analysis show that the assembled systems might fall into one of three phases depending on Ca and gamma: (1) loosely connected network of F-actin and bundles, (2) loosely connected network of dense domains and (3) uniform network of bundles. The phenomena can be explained statistical mechanically and replicated using our MD simulations.
We have also carried out simulations with different types of cross-linkers to represent the proteins, fascin and filamin. Our results show that the formation of different phases is related to the flexibility in binding between F-actin and cross-linkers. This degree of freedom, possible with a longer cross-linker allows the possibility of forming branching points and thus bundle networks (The alpha-actinin/actin and filamin/actin systems form bundle networks, but the fascin/actin system does not).

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Cooperative Interactions Between Myosin II and Actin Cross-Linking Proteins To Actin Filaments

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Heavy meromyosin (HMM) of both muscle myosin II and non-muscle myosin II cooperatively binds to actin filaments in a manner that depends on divalent ion composition and concentration or the transition state of the myosin motor domain. Therefore, the cooperative binding may be due to the conformational changes in F-actin induced by two-headed HMM that facilitate the binding of other heads to nearby regions in the same actin filament. Recently, we found that myosin II and an actin crosslinker cortexillin I cooperatively accumulate to highly deformed regions in Dictyostelium cells and the accumulation extent increases with increasing forces. One possible mechanism of the cooperative accumulation is that the binding of myosin to actin enhances cortexillin binding to actin filaments. We suspect this kind of cooperative binding might also exist among other actin crosslinkers. We are testing this hypothesis by using novel designed in vitro systems and advanced molecular simulation schemes. We are experimentally investigating this mechanism by measuring the binding lifetimes of myosin II and actin crosslinkers to actin and their accumulations at different load conditions in reconstituted actin cytoskeletons. One of the reconstituted systems is a bilayer vesicle that has an actin cortex anchored to its inner lipid membrane. Another system is an actin meshwork crosslinked by various actin crosslinkers and stiffened by myosin II. It is positioned in a motor-driven stretcher, allowing for in situ TIRF microscopic measurements at different strains and different strain rates. In addition, we simulate the corresponding two-dimensional reaction-diffusion problems using coarse-grained kinetic Monte Carlo simulation. In our simulations, the diffusion coefficients vary in the range of 0.01~100 μm2/s and the forward and backward characteristic time of the binding reactions is in the range of 100 s and 0.01 s.

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Turnover Dynamics of Diffuse Actin and Regulators At the Leading Edge

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The lamellipodium at the leading edge of motile cells is a dynamic structure consisting of a dense network of branched actin filaments. These actin filaments polymerize in a region close to the leading edge and undergo retrograde flow towards the main body of the cell. A large number of experimental techniques have been used to monitor the structural aspects of actin networks at the leading edge. However, the role of concentration gradients and local heterogeneity of